Methods: Actfl/fl mice were crossed with a ubiquitously expressed inducible Cre recombinase line (UbiCreERT2). Male and female mice homozygous for the floxed allele and positive for Cre recombinase were treated with tamoxifen at 8 weeks of age to induce deletion of the gene in all tissues. Actfl/fl mice (without Cre) were used as negative controls. OA was induced by destabilization of the medial meniscus. 8 weeks following surgery joints were taken for microCT analysis to measure epiphyseal volume according to our previously published method (Das Neves Borges et al, 2014) and serial histology for assessment of cartilage degradation.
Results: No overt deleterious effect of global post natal activin bA deletion was observed. Knockout mice were noted to have delayed hair regrowth following shaving (prior to surgery). Cartilage degradation scores showed a trend towards being lower in knockout compared with wild type animals although this did not reach statistical significance. Epiphyseal volume, a validated measure of osteophyte size, was significantly reduced in female, but not male, knockout mice. By histology, osteophytes were present in female knockout mice but were very small compared with male knockout animals or the wild type controls.
Conclusions: We have uncovered a novel role for activin bA in the development of osteophytes following joint destabilization that is restricted to female mice. Although there was a trend towards reduced cartilage degradation scores in knockout animals, these did not reach significance.

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IGF-II INHIBITS PROMOTES CARTILAGE INTEGRITY IN EXPERIMENTAL OSTEOARTHRITIS AND IL-1β-INDUCED CATABOLIC ACTIVITY IN CHONDROCYTES

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Purpose: The goal of this study was to investigate the effect of IGF-II on chondrocyte catabolic gene expression under pro-inflammatory cytokine IL-1β treatment and on cartilage matrix levels in OA.
Methods: IN VITRO: Normal human articular chondrocytes (Lonza) were cultured in the presence or absence of IL-1β or lentiviral hIGF-II or GFP, and subjected to RT-PCR and Western blot analysis. IN VIVO: CD1 mice were subjected to DMM (destabilization of medial meniscus) surgery. Lenti-IGF-II or GFP were intraarticularly injected at 1 and 2 weeks post DMM or sham surgery (6 mice/treatment). Safranin O staining and Immunohistochemistry (IHC) were performed on sectioned knees and scored by Image J and the OARSI scoring system. EX VIVO: human OA cartilage slices were cultured in the presence of IGF-II (10ng/ml) for 3 weeks and matrix level was analyzed by IHC and safranin/O staining. Data are reported as mean ± standard deviation. For parametric data, one-way analysis of variance followed by post-hoc Tukey test was used. For nonparametric data (OARSI scores), we used Kruskal-Wallis test followed by Mann-Whitney U test with Bonferroni correction. P-values of <0.05 were considered significant.
Results: To ascertain IGF expression in OA cartilage, we performed RT-qPCR analysis using cartilage specimens obtained from the tibial plateaus of a cadaveric donor and three OA patients undergone total knee replacement surgery. We found that the expression of IGF-II, but not IGF-I, was reduced in OA cartilage, while IL-1β and MMP13 were increased in OA (Data not shown). We then tested whether IGF-II could antagonize IL-1β activity in primary human articular chondrocytes, and found that co-treatment of IL-1β with IGF-II restored Col-II and Col-IX expression and inhibited Col-I, MMPs and iNOS induction (Fig. 1A). Furthermore, treatment of IL-1β with IGF-II significantly inhibited NF-κB nuclear localization and IκBα degradation (Fig. 1B), as well as NFATc2 nuclear localization (data not shown). As phospholipase C (PLC) has been reported to act downstream of IGF-I and IGF-II in chondrocytes, we used a PLC inhibitor U73122, and found that IGF-II no longer prevented IL-1β-induced MMP13 expression upon U73122 treatment. In contrast, an inactive analog of PLC inhibitor, U73343, could not prevent IGF-II from inhibiting MMP13 expression (Fig. 1C), suggesting that PLC mediates IGF-II activity with this respect.
To determine the effect of IGF-II on articular cartilage in OA in vivo, we utilized the DMM mouse model, where we found IGF-II expression to be decreased in the DMM knee joint as compared to the sham (Fig. 2A). We then ectopically expressed IGF-II in mouse OA joints through intra-articular injection of lentiviruses encoding IGF-II or GFP (control) at 1 and 2 weeks after the surgery. The efficiency of viral transduction was assessed by IHC (data not shown), and joint integrity was analyzed by

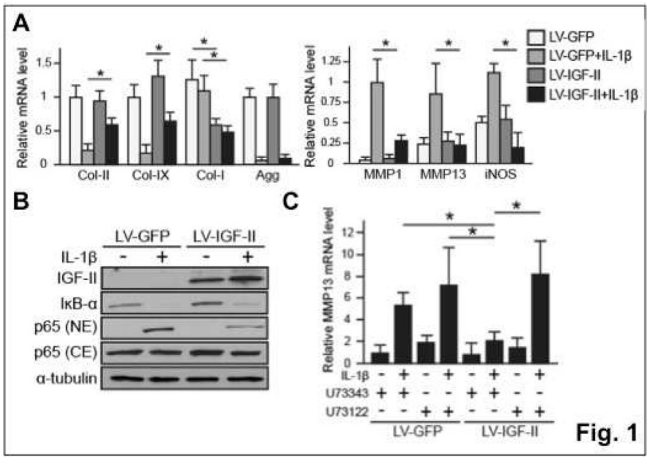
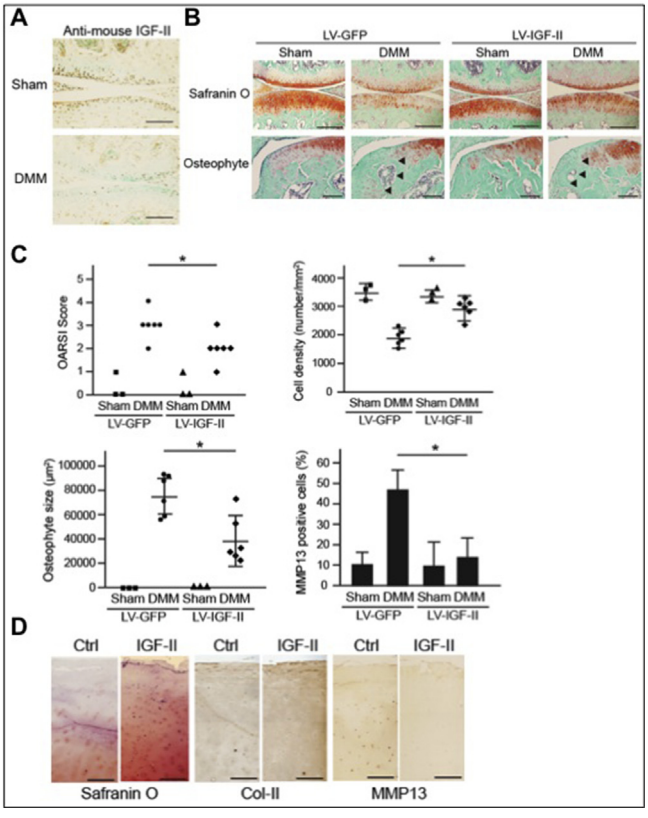


Fig. 1

Safranin O staining. No significant differences were observed between lentiviral-IGF-II or GFP treated sham knees. However, lentiviral-IGF-II injected DMM knee joints retained more Safranin O staining and had smaller osteophyte formation compared to DMM joints injected with the GFP virus (Fig. 2B and 2C). Furthermore, ectopic IGF-II expression significantly reduced MMP13 and NFkB (p65) expression in the DMM knee joints (Fig. 2C and data not shown). Consistent with the mouse data, we also found that IGF-II led to stronger safranin/O staining and higher levels of Col-II expression, as well as reduced levels of MMP13 expression in human OA cartilage explants (Fig. 2D). Although it is unclear whether IGF-I has a similar activity as IGF-II in OA, explant cultures using IGF-II deficient mouse metatarsal bones indicated that IGF-II, but not IGF-I, could rescue IL-1β-mediated cartilage matrix damage, suggesting of a functional difference between the two growth factors (data not shown).
Conclusions: 1. IGF-II inhibits IL-1β-induced matrix gene reduction and NF-κB and NFAT activation in human articular chondrocytes in vitro, and its activity is mediated by PLC.



2. IGF-II is capable of promoting cartilage matrix maintenance in injury-induced OA in mice and in human OA cartilage ex vivo cultures.

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CHANGES IN PATELLOFEMORAL BONE MARROW LESIONS AND KNEE PAIN: NATURAL HISTORY AND THE ASSOCIATIONS WITH STRUCTURE

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Purpose: Bone marrow lesions (BMls) are recognized as an important subchondral feature in knee OA and play a vital role in the diease progression. The patellofemoral joint (PFJ) is a common site of pain and contributes to functional limitations among OA patients. However, there are very few clinical or epidemiological studies that reveal the association between PFJ BMLs and clinical symptoms as well as cartilage structural morphologies. Meanwhile, the natural history of PFJ BMLs has not yet been described. The aims of this study were, therefore, to describe the natural history of MRI-detected BMLs in PFJ over 2.6 years and evaluate the association between increases in PFJ BMLs, knee pain and knee cartilage morphology in older adults.

Methods: 406 males and females were randomly selected from local community (mean age 63 years, range 51 to 79) and followed up for 2.6 years. PFJ BMLs were determined on T2-weighted fat saturated magnetic resonance imaging (MRI) using Whole-Organ MRI Score system (WORMS). Knee cartilage volume and cartilage defects scores (0-4) were determined on T1-weighted fat suppression MRI using WORMS. Knee pain was accessed by Western Ontario and McMaster Universities Osteoarthritis (WOMAC) scores. Student's t-tests and Pearson's χ^2 tests were used to compare the differences between subjects with and without an increase in PFJ BMLs. Crude and adjusted linear regression was used to determine whether PFJ BML changes over 2.5 years were associated with changes in knee pain in the different sub-scales over 5 years, before and after adjustment for potential confounders. Binary logistic regression was used to examine the associations between increases in PFJ BMLs as an outcome, and baseline cartilage volumes as well as baseline cartilage defect scores as predictors, both before and after adjustment for potential confounders.

Results: At baseline, 27% (n=109) had PFJ BMLs, 24% of these showed progression (change in score of ≥ 1) at follow-up, 44% persisted and 21% completely resolved. Of those 73% (n=297) who did not have PFJ BMLs at baseline, 19.7% of them developed new PFJ BMLs over 2.6 years. In multivariable analysis, change in PFJ BMLs was positively associated with increases in total knee pain (β: 0.81, 95% CI: 0.15, 1.48) and knee pain when going up/down stairs (β: 0.29, 95% CI: 0.08, 0.50) over 5 years. While baseline patellar cartilage volume predicted a decrease in PFJ BMLs (OR: 0.62, 95% CI: 0.43, 0.90), baseline patellar cartilage defects were associated with an increase in PFJ BMLs (OR: 1.75, 95% CI: 1.28, 2.40) over 2.6 years. Tibiofemoral cartilage volume and defects were not associated with changes in PFJ BMLs.

Conclusions: PFJ BMLs are not static and change is clinically relevant. An increase in PFJ BMLs can be predicted by reduced patellar cartilage volume and increased patellar cartilage defects site-specifically.

	Patellofemoral joint BMLs		
	No Increase (n=321)	Increase (n=85)	*p* value
Age	62.9±7.1	61.8±7.6	0.246
Females (%)	50	50	0.863
BMI (kg/m^2)	27.5±4.3	28.0±4.9	0.382
ROA present (%)	70	61	0.754
Knee pain (%)	50	40	0.541
Smoked (%)	50	40	**0.037**
Tibia Bone size (cm^2)	33.6±4.9	32.7±4.2	0.133
Patella BMLs at baseline (%)	18	19	0.873
Tibiofemoral BMLs at baseline (%)	32	42	0.086
Patella Cartilage defect score at baseline	1.5±0.9	1.9±1.0	**0.001**
Tibiofemoral Cartilage defect score at baseline	4.2±1.8	4.4±1.6	0.372
Patella cartilage volume at baseline (ml)	3.4±0.9	3.2±0.9	**0.031**
Tibia cartilage volume at baseline (ml)	5.1±1.2	5.0±1.0	0.291

Abbreviation: ROA, radiographic osteoarthritis; BMI, body mass index; BMLs, bone marrow lesions. *Data are given as mean ± SD unless otherwise indicated. Student's t-test or chi-square test (where appropriate) were used to test for significant differences between two groups. An increase in patellofemoral joint (PFJ) BMLs is defined as a change in BMLs of ≥1 from baseline to follow-up (vs. no increase).

	Univariable β (95% CI)	Multivariable * β (95% CI)	Multivariable** β (95% CI)
Total WOMAC knee pain	**0.78 (0.15, 1.40)**	**0.81(0.17, 1.45)**	**0.79 (0.11, 1.47)**
Pain walking on a flat surface	0.03 (-0.12, 0.18)	0.03 (-0.12, 0.19)	0.05 (-0.11,0.22)
Pain going up and down stairs	**0.28 (0.07, 0.48)**	**0.27 (0.07, 0.47)**	**0.29 (0.08, 0.50)**
Pain at night when in bed	**0.22 (0.03, 0.41)**	**0.22 (0.02, 0.42)**	0.19 (-0.02, 0.40)
Pain sitting or lying	0.12 (-0.02, 0.27)	0.13 (-0.02, 0.28)	0.11 (-0.05, 0.27)
Pain standing upright	0.13 (-0.02, 0.29)	0.15 (-0.01, 0.32)	0.15 (-0.01, 0.32)

*Adjusted for age, sex, BMI, ROA, NSAIDs use and smoking status, **further adjusted for baseline patellofemoral BML, baseline tibiofemoral BMLs, baseline patella cartilage defect and baseline tibiofemoral cartilage defect. Statistical significances are shown in bold.

	Univariable RR (95% CI)	Multivariable* RR (95% CI)	Multivariable** RR (95% CI)
Baseline patella cartilage volume (ml)	**0.78 (0.62, 0.99)**	**0.68 (0.50, 0.92)**	**0.69 (0.48, 0.98)**
Baseline Tibial cartilage volume (ml)	0.94 (0.83, 1.07)	0.86 (0.69, 1.07)	0.87 (0.70, 1.08)
Baseline patella cartilage defects (per grade)	**1.35 (1.13, 1.60)**	**1.50 (1.19, 1.90)**	**1.67 (1.26, 2.19)**
Baseline Tibiofemoral cartilage defects (per grade)	1.04 (0.96, 1.13)	1.03 (0.93, 1.14)	0.99 (0.89, 1.10)

*Adjusted for age, sex, BMI, baseline patella BMLs, smoking status and ROA. ** further adjusted for baseline PFJ BMLs, baseline total tibiofemoral BMLs, total cartilage defects and total cartilage volume. Significant differences are shown in bold.

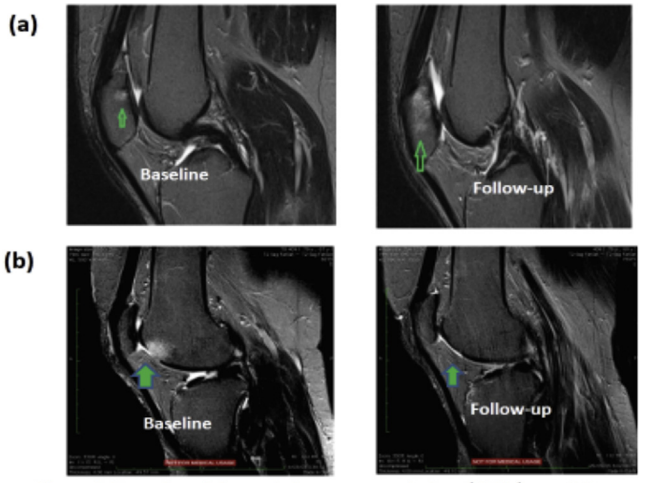

Figure 1 Examples of change in bone marrow lesions (BMLs) over 2.6 years.
(a) PFJ BMLs increase from baseline to follow-up.
(b) PFJ BMLs completely resolved from baseline to follow-up.